



## RESEARCH ARTICLE

# Morphology, histology and histochemistry of the digestive tract of the Banded tilapia, *Tilapia sparrmanii* (Perciformes: Cichlidae)

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**ABSTRACT.** This study described anatomical, histological and histochemical features of the mucosal layer of the digestive tract of *Tilapia sparrmanii* Smith, 1840, an omnivorous freshwater fish endemic to Southern Africa. This species exhibited a short thick oesophagus with long deep longitudinal folds ( $466.68 \pm 16.91 \mu\text{m}$ ), and a thick ( $173.50 \pm 10.92 \mu\text{m}$ ) muscular layer that allow the passage of large food items. The mucosa was lined with stratified secretory epithelium rich in goblet cells that secreted neutral and acid mucins. The stomach was a sac-like structure with simple tubular glands surrounded by connective tissue. The mucosa was lined with simple columnar epithelium and the lamina propria exhibited a well-developed layer of gastric glands that occupied the entire length of the cardio-fundic region. The stomach mucosa consisted of epithelial cells with intense neutral mucin secretion which protects against gastric juice. Neck cells of gastric glands synthesized neutral and acid mucins. The intestine was highly coiled and presented a complex pattern of transversal folds internally (villi). Villi length decreased progressively from the anterior to the posterior intestine ( $p < 0.0001$ ). Tunica muscularis of the mid-intestine had the thinnest thickness among all parts of the intestine ( $p < 0.0001$ ). Goblet cells whose numbers increased towards the rectum secreted both acid and neutral mucins. The results indicate structural similarities of *T. sparrmanii* GIT with other tilapia species and will be useful for understanding the physiology of the digestive systems as well as functional components of the GIT.

**KEY WORDS.** Fish, gastrointestinal tract, histo-architecture

## INTRODUCTION

The basic plan of the gastrointestinal tract (GIT) of fish is similar to that of other vertebrates (Wilson and Castro 2010). The anatomical and histological structures of fish GIT, on the other hand, show a marked diversity, which reflects the phylogeny, ontogeny, diet, and environment (Kozarić et al. 2007, Hernández et al. 2009, Santos et al. 2015, Xiong et al. 2011). Normally, carnivorous fish have a large and distensible stomach, and a short intestine (Qu et al. 2012). Omnivores that eat greater amounts of animals in their diet have a large stomach and a longer intestine. Omnivores that eat a great amount of plants typically have a smaller stomach and a long intestine. Herbivorous fish characteristically have either a small or no stomach at all, and the longest and most complex intestine (Smith 1991).

The GIT in fish plays a critical role in growth, nutrition and survival of the fish under various environmental conditions. The mouth serves to capture and pre-process food before it enters the oesophagus. The latter is a short and muscular, mucous-secreting connection leading to the stomach. The stomach, when present,

is the portion of the GIT that displays most variations (Musa et al. 2013, Gosh and Chakrabarti 2015a, 2015b), and serves the purpose of food digestion. The intestine is the main organ for nutrient absorption (Sanchez-Amaya et al. 2007, Smith et al. 2000), usually aided by the variable finger-like appendages, the pyloric caeca, present between the stomach and intestine. Undigested food is often expelled through the anus, aided by numerous mucous secreting cells located in the distal intestine and rectum.

The ability of fish to utilize ingested nutrients depends on the presence of appropriate enzymes in appropriate locations in the wall and along the lumen of the GIT. Mucus helps to protect epithelial surfaces against mechanical damage while enabling rapid removal of various types of aggressive pathogens as well as irritants (Reid et al. 1988, Roussel and Delmotte 2004); and forms a diffusion barrier for various ions between the luminal content and epithelial lining (Vegetti et al. 1999). The histological architecture of the GIT in fish includes a layer of mucus secreting cells, which have been described extensively in most teleost fish by histochemical techniques (Domeneghini et al.



2002, Kozarić et al. 2007, Leknes 2010, 2011, Musa et al. 2013, Gosh and Chakrabarti 2015a, 2015b, Wołczuk et al. 2015). However, under specific environmental conditions, and particularly as questions that engage scientists expand, the best focus may involve examination of little studied non-model organisms.

The GIT is one of the major organ systems of fishes that interacts with the environment and plays a critical role in growth, nutrition, as well as survival under stressful environments. Further, several functions of various segments of the GIT are controlled by endocrine cells, which can vary in frequency and distribution depending on the fish and prevailing environmental conditions (Cinar and Senol 2006, Diaz et al. 2003). Therefore, for better understanding of mechanisms underlying morphological diversification, broader sampling at different geographical settings is crucial.

*Tilapia sparrmanii* Smith, 1840, commonly known as Banded tilapia (Dunz and Schliewen 2010, 2013), is a benthopelagic, potamodromous species widely distributed in rivers, reservoirs and swamps in many parts of Central and Southern parts of Africa (Teugels et al. 1991, Gonzales and Brown 2007, Skelton, 1993). They play an important role in the food chain; are also used as bait and often preyed upon by large fish species (Dunz and Schliewen 2010). However, habitat degradation, pollution and destruction of breeding grounds are major threats to this fish species in its natural habitat. *Tilapia sparrmanii* has potential for aquaculture and laboratory use. However, there is limited information in the literature on their digestive physiology including distribution of mucins throughout the GIT, which play a role on the regulation of digestive processes. Insights into the anatomy of the digestive system of an aquaculture candidate fish are also of importance in guiding development of appropriate feeding strategies of the fish once cultured. Therefore, in the present study we describe morphological, histological and histochemical aspects of the GIT of *T. sparrmanii* with the aim of providing background information that would help in the understanding of its feeding physiology, and also to evaluate the use of GIT barrier integrity as an organ for testing the impact of various environmental stressors.

## MATERIAL AND METHODS

Fish were collected from a freshwater dam in Mthatha in the Eastern Cape Province, South Africa ( $31^{\circ}32'S$ ;  $28^{\circ}43'E$ ) during routine sampling. Twenty-one adult specimens of *T. sparrmanii*, body mass about 0.32–0.40 kg, 110–120 mm total length (TL) were used in the current study. Fish were sacrificed by immersing in an overdose of tricaine methane sulphonate (MS-222, Sigma Chemicals, MO, USA). The whole GIT; oesophagus, stomach (glandular and non-glandular), and three intestinal segments (anterior, middle and posterior) was explanted from each specimen (Figs 1–3) and fixed in Histochoice fixative overnight, rinsed with distilled water and stored in 70% ethanol. Fragments of the oesophagus, stomach (glandular and non-glandular) and three intestinal regions (anterior, mid-intestine and posterior) were then dehydrated through ethanol series, cleared in HistoChoice® (Sigma-Aldrich,

St. Louis, MO, USA) and embedded in Paraplast® (Merck, Darmstadt, Germany). Consecutive longitudinal and transverse sections (4–6  $\mu\text{m}$  thick) of segments of the GIT of each fish were cut and mounted on slides coated with 3-amino-propyl-triethoxy saline (Sigma-Aldrich, St. Louis, MO, USA). Ten slides obtained from each specimen ( $n = 6$ ) were prepared for each of the three staining procedures: haematoxylin and eosin (H&E) or periodic acid Schiff (PAS) for neutral mucins and alcian blue (AB pH 2.5) for acidic mucins. HistoChoice products were preferred in the current study because they are odourless, and much less hazardous compared to commonly used solvents such xylene and toluene. Furthermore, the research reported in this article was part of a broader project that required immunohistochemical studies as well. These products are known to better preserve antigenic sites for antibody probes and nucleic acid sites for immunohistochemistry.

Histochemical procedures comprised periodic acid Schiff (PAS). PAS staining was done according the packet insert and according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA) with or without haematoxylin.

PAS and Alcian blue (AB) pH 2.5 staining was done to reveal neutral and acid mucins, respectively. Here tissue sections of the GIT were deparaffinized and hydrated to distilled water, incubated in 3% acetic acid. Thereafter, sections were stained in alcian blue for 30 minutes and washed in running tap water. Tissue sections were counterstained in nuclear fast red for three minutes, rinsed in tap water and dehydrated. Sections were cleared in HistoChoice® clearing agent and mounted with Leica CV mount (Leica Microsystems Nussloch, GmbH).

Prepared slides were used for the measurement of individual thickness of the layers making up the wall of the GIT. For each of these parameters, 20 measurements were taken of which an average was calculated. Results were presented as means  $\pm$  SD values. Intensity of histochemical labelling with PAS and AB pH 2.5 was evaluated semi quantitatively according to observed intensity of color reaction, i.e., (–) no staining, (+) low, (++) medium, and (+++) strong staining (Table 1). Images of stained material were acquired using a Leica DM 750 microscope (Leica Microsystems, GmbH, Germany), attached to a DFX 310 FX digital camera.

All measurements were performed using Leica LAS imaging software version 4.5, (magnification 10x). Means of various parameters of intestinal segments was compared using one-way ANOVA. Scheffe's test was used to compare values between the segments using Statistical Package of Social Science (SPSS), version 13. The significance level was set at 0.0001.

Experimental protocols for the study were approved by the Animal Ethics Screening Committee, Faculty of Natural Sciences, Walter Sisulu University.

## RESULTS

The Gross morphology of gastrointestinal tract (GIT) in *T. sparrmanii* (Fig. 1), like other typical teleost, consists of a short oesophagus, which is attached to the stomach. The stomach itself was sack-like structure connected to oesophagus and proximal

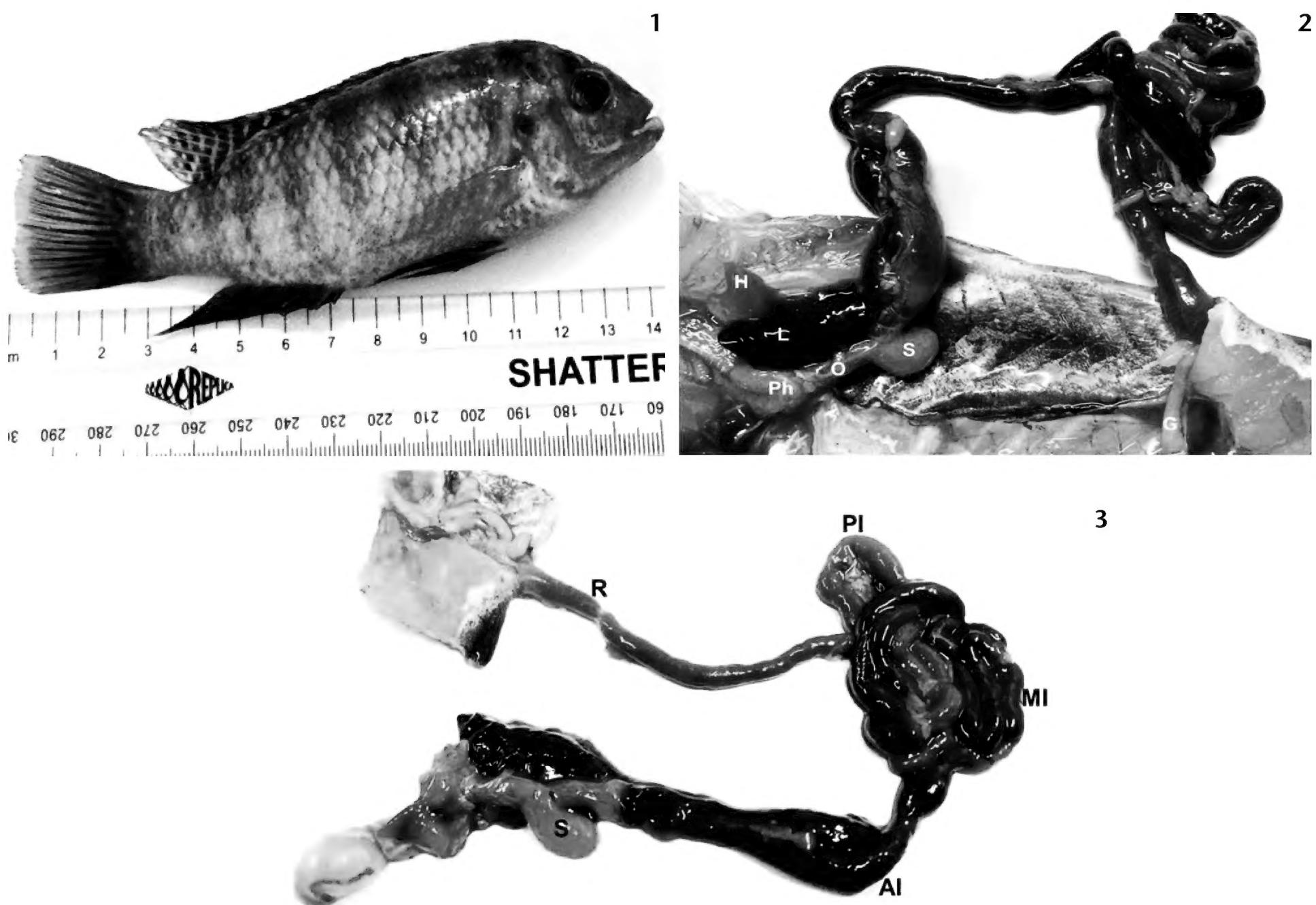
region of the intestine (Fig. 2). Morphologically, the intestine was tubular, long and highly coiled and was divided into three segments; the anterior, mid region (mid-intestine) and posterior (rectum). The size of the intestine was not uniform through the entire length wherein; the proximal intestine was larger than the rest of the intestine (Fig. 3).

Histologically the GIT of *T. sparrmanii* follows the general plan as in other vertebrates, consisting of four layers: the mucosa,

sub-mucosa, muscularis and serosa. The three segments of the intestine had the same basic organization of the muscular layer as in the stomach, consisting of an external longitudinal and internal circular muscle layers.

#### Oesophagus

Microscopically the mucosa of the esophagus exhibited deep longitudinal folds which extended the entire length of



Figures 1–3. (1) Image of an adult of *Tilapia sparrmanii* in the lateral view; (2) gross morphology of the gastrointestinal tract (GIT) of *T. sparrmanii* in ventral view of fish showing the relationship of the gastrointestinal tract with other organs in the abdominal cavity. Oesophagus (O) connected to pharynx (Ph) and stomach (S), which overlapped by the liver (L) and heart (H). Notice the highly coiled intestine (I). (3) Gross morphology of the gastrointestinal tract (GIT) of *T. sparrmanii*, showing the stomach (S). The intestine is divided into anterior intestine (AI), middle intestine (MI), posterior intestine (PI) and rectum (R).

Table 1. Intensity of histochemical labeling of mucins in the GIT of *T. sparrmanii*. (AB) Alcian blue, (ES) Epithelium surface, (PAS) periodic acid Schiff.

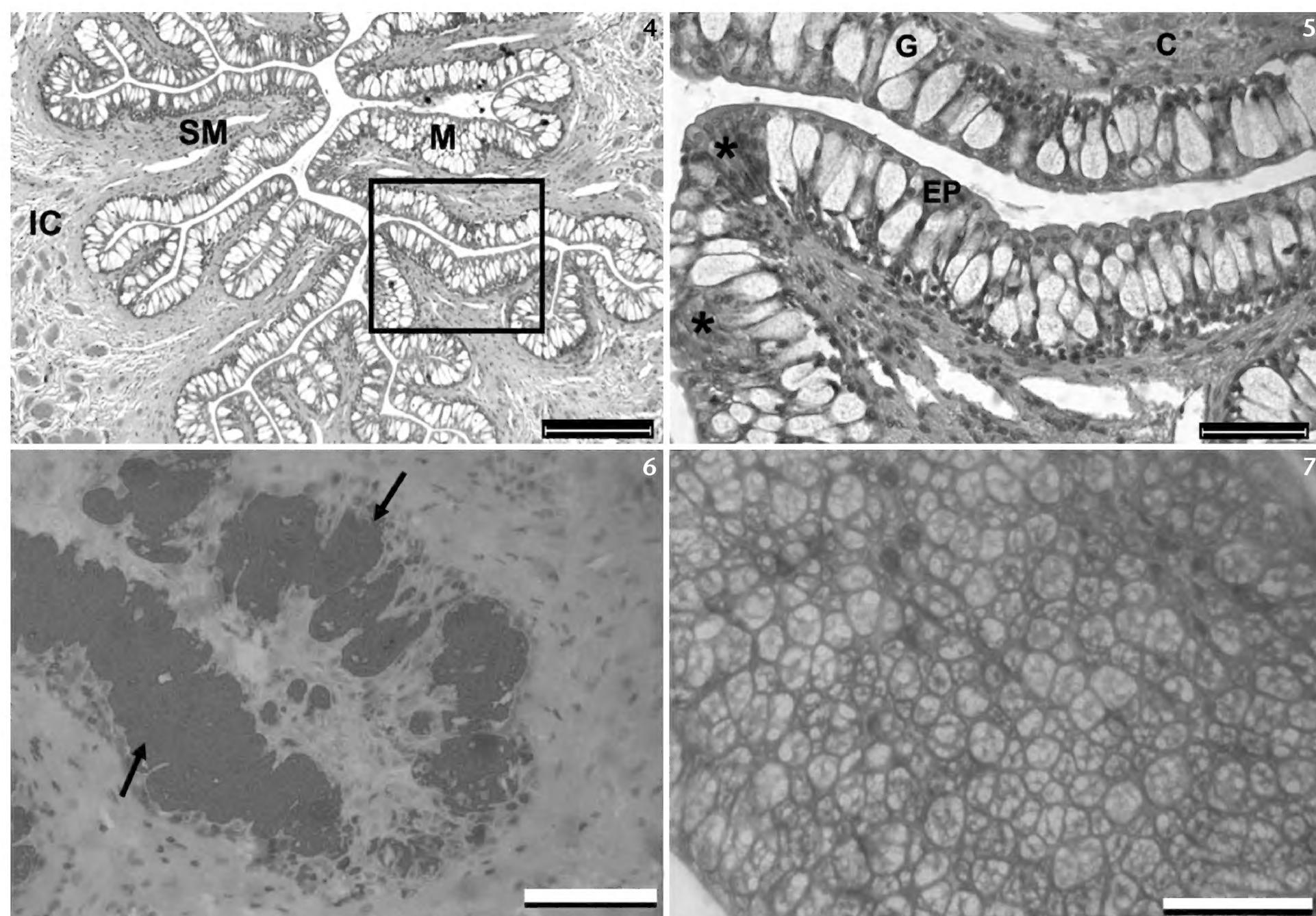
Procedure	Oesophagus			Stomach			Anterior intestine			Mid intestine			Posterior intestine			
	ES	Goblet cells		ES / Neck cells	Goblet cells		ES	Goblet cells		ES	Goblet cells		ES	Goblet cells		
		Reaction	Distribution		Reaction	Distribution		Reaction	Distribution		Reaction	Distribution		Reaction	Distribution	
Pas	+	++	+++		+++ / ++	++	++	-	+++	+++	+	+++	++	+	+++	+++
Ab ph 2.5	+	+++	+++		- / ++	-	++	-	+++	+++	+	++	++	+	++	+++

Intensity: (-), no staining observed; (+), low; (++) medium; (+++), strong.

the oesophageal tube (Fig. 4); mean fold length of  $466.68 \pm 16.91 \mu\text{m}$  and fold width of  $176.32 \pm 15.40 \mu\text{m}$ . The epithelium was stratified squamous with epithelial cells interspersed with two types of cells; basal, superficial epithelial cells. The cells at the surface acquired squamous aspect and rounded nuclei (Figs 4, 5). The most prominent feature of the oesophagus was the presence of numerous mucous cells of different sizes, which were arranged at different levels of the epithelium and occupied almost the entire thickness of the epithelium (Figs 4, 5). Small mucous cells were seen at the surface and large ones extended to the base of the epithelium. Other surfaces of the epithelium were devoid of mucous cells (Fig. 5). The lamina propria was composed of connective tissues, while the sub-mucosal layer consisted of dense connective tissue and blood vessels (Figs 1, 2). The muscular layers were characterized by loose connective tissue and depicted striated muscular

fibres arranged longitudinally on the inner and in a circular manner on the outer layer mean muscular layer of  $173.50 \pm 10.92 \mu\text{m}$ . The serosa was well developed and consisted of dense connective tissue. Histological sections indicated a distinction of the transition from the oesophagus to the stomach. This feature was characterized by the replacement of the stratified squamous epithelium with mucous cells to a simple columnar epithelium of the stomach.

The distinct feature of mucosal epithelium was that it was made up of stratified epithelium consisting of basal cuboidal cells, intermediate columnar mucous cells and superficial layer of flattened cells (Figs 4, 5). The numerous columnar mucous cells which were present in entire mucosal epithelium constituted its major thickness which stained positive with Alcian Blue (pH 2.5) (Table 1, Fig. 6) and Periodic Acid Schiff (Table 1, Fig. 7).



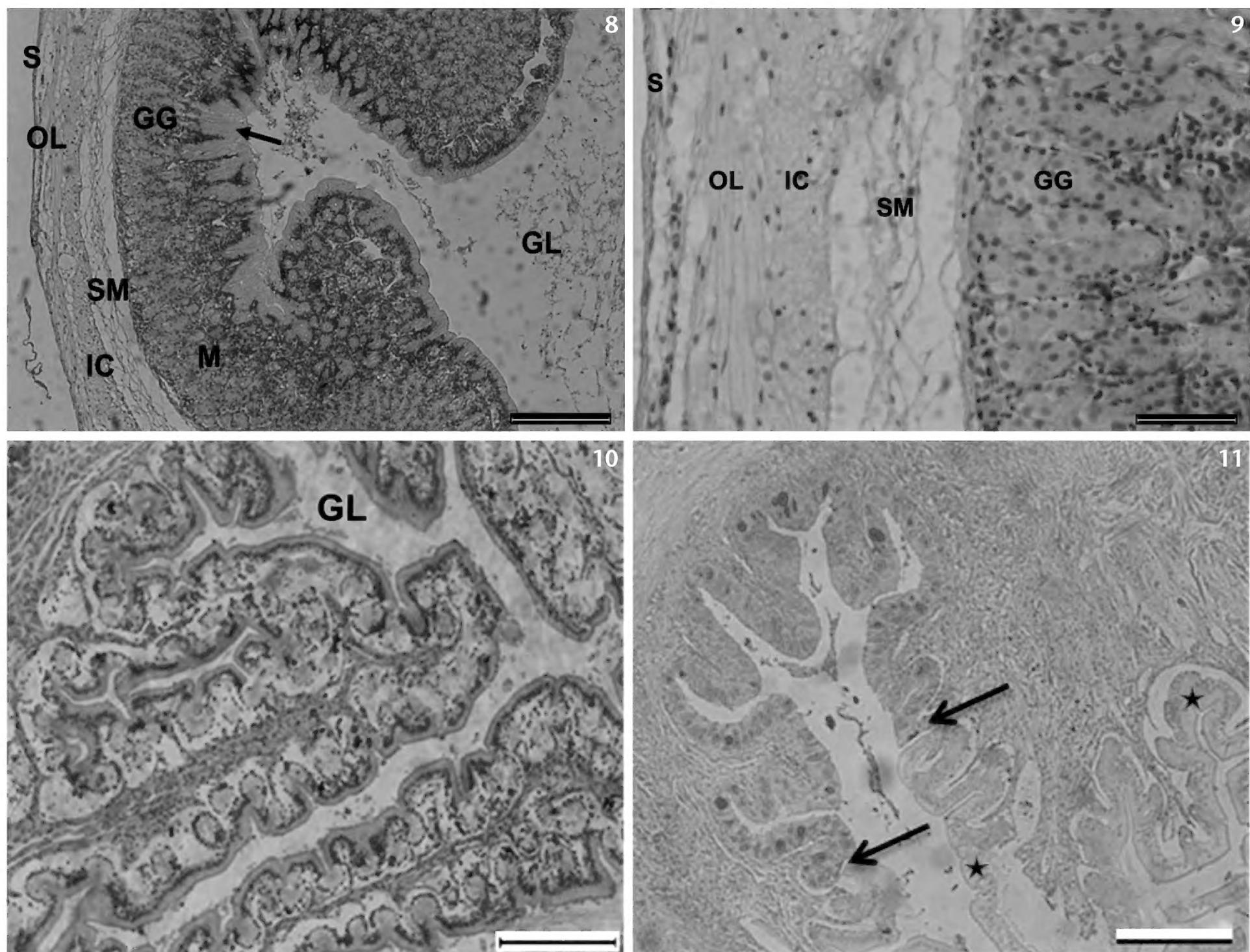
Figures 4–7. (4) Photomicrograph of the oesophagus of *T. sparrmanii* showing distinct layers; mucosa (M), submucosa (SM), muscularis which consisted of inner circular (IC) and outer linear and a serosa. H&E stain. (5) An enlarged transverse section of the area marked by box in Fig. 4, showing the epithelial lining of the oesophagus (EP) with mucus secreting cell (G) and connective tissue core (C). Note the absence of mucus cells in some regions of the epithelium (\*). H&E stain. (6) Transverse section of the oesophagus of *T. sparrmanii*, showing AB (pH 2.5) positive cells (arrows). (7) Transverse section of the oesophagus of *T. sparrmanii*, showing PAS positive cells. PAS/haematoxylin stain. Scale bars: 4 =  $20 \mu\text{m}$ , 5–7 =  $50 \mu\text{m}$ .

### Stomach

The stomach in *T. sparrmanii* is morphologically differentiated into two regions: the glandular (cardiac and fundic) and non-glandular (pyloric) regions (Figs 8–10). The cardiac was the most prominent of the regions in terms of cellular structures (Figs 8, 9). The wall of the cardiac region consisted of the inner most, the mucosa, made up of two types of epithelia; an outer superficial and the inner glandular (Figs 8, 9). The superficial epithelium was made up of compactly arranged single layer of columnar epithelial cells with basally located conspicuous nuclei. Luminal cells surrounded the gastric lumen and gastric pits. These cells possessed homogenous finely granulated cytoplasm and rounded

basally located nuclei. The glandular epithelium consisted of numerous gastric glands (GGs) that occupied the entire mucosa just beneath the superficial epithelium. They were made up of rhomboid shaped cells which exhibited centrally located nuclei and opened up into the lumen of the stomach via gastric pits (Fig. 8). Within the mucosa, GGs were tightly packed jointed by thin threads of connective tissue. Beneath the epithelium was the lamina propria of the cardiac region of the stomach, which consisted of glandular and loose connective tissue layers that held the GGs and penetrated the mucosal folds (Figs 8, 9).

The lumen of the pyloric stomach was narrow due to the extensive development of rugae; mean rugae width and depth



Figures 8–11. (8) Photomicrograph of the cardiac stomach of *T. sparrmanii* showing mucosal fold consisting of lamina propria with numerous gastric glands (GG), and muscularis, which consisted of inner circular (IC) and outer longitudinal (OL), the serosa (S) and the epithelial layer with gastric pits (arrow). H&E stain. (9) An enlarged photomicrograph of the cardiac stomach of *T. sparrmanii* showing the different layers; the sub mucosa (SM), muscularis consisting of inner circular (IC) and outer longitudinal (OL) muscle layers, the serosa (S) and gastric glands (GG). H&E stain. (10) Photomicrograph of the pyloric stomach of *T. sparrmanii* showing villi like projections into the gastric lumen. Note the absence of gastric glands. (11) Photomicrograph showing the transition between the oesophagus and the stomach (arrows). Note the absence of AB (pH 2.5) positive cell in the pyloric region of the stomach (\*). Scale bars: 8, 10, 11 = 200 µm, 9 = 50 µm.

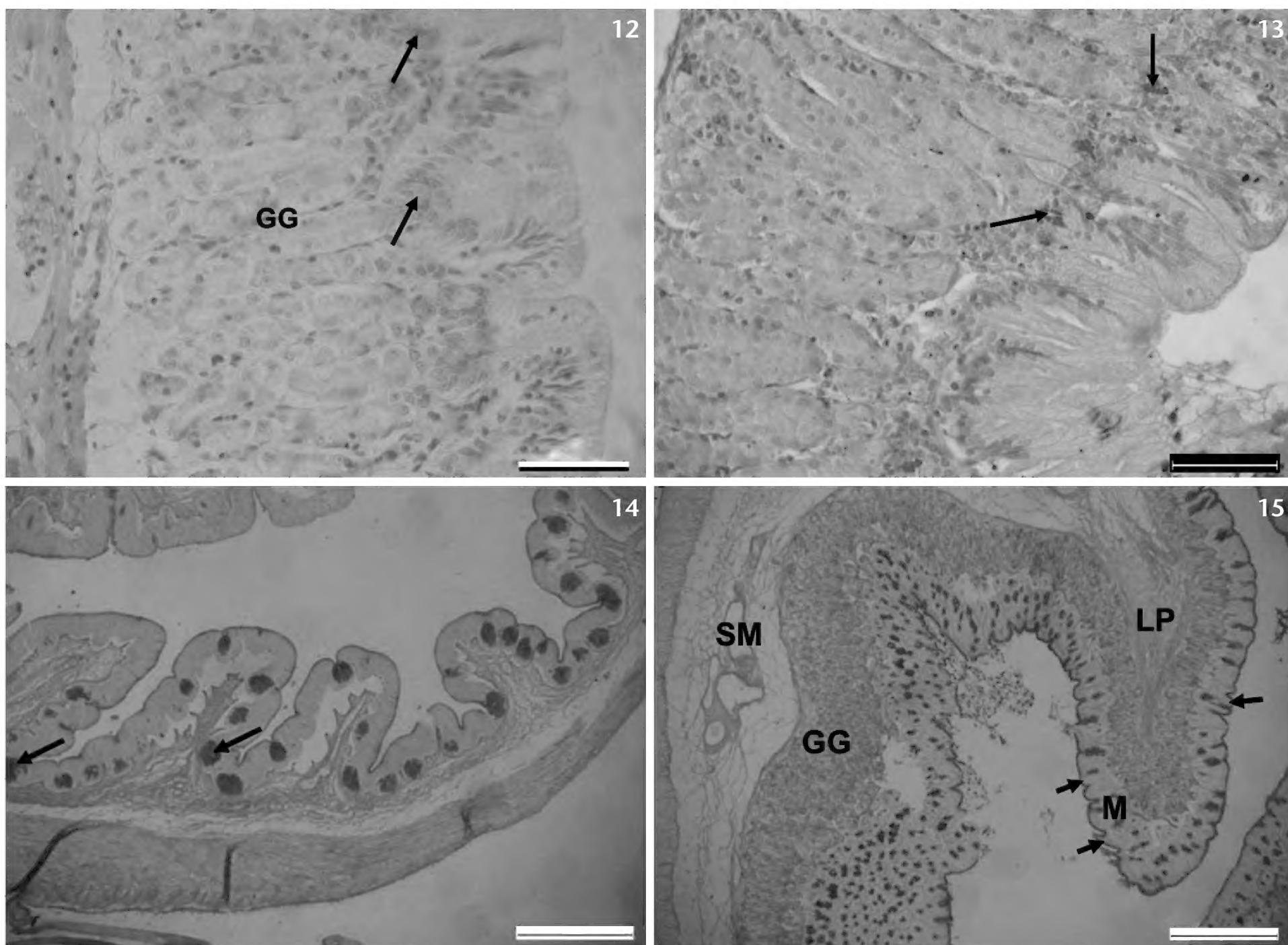
of  $304.29 \pm 35.27 \mu\text{m}$  and  $686.87 \pm 76.54 \mu\text{m}$  respectively (Fig. 10). This region of the stomach was devoid of GGs and was characterized by having numerous fingerlike villi lined with single layer of columnar epithelial cells with deep darkly stained nuclei and mucous cells.

The sub-mucosa of the stomach was thin and highly vascularized, composed of a network of connective tissue, collagen, including blood vessels, which projected into the mucosal folds forming the lamina propria (Figs 8, 9). The tunica muscularis was made up of smooth muscle fibres arranged in two layers; inner circular and outer longitudinal muscle layers, mean muscle tissue layer of  $151.03 \pm 16.07 \mu\text{m}$  (Figs 8–10). The inner circular layer of the tunica muscularis was thicker than the outer layer. The serosa was very thin and formed the outermost layer of the stomach. Cells of the serosa were small and flattened with homogenous cytoplasm and compressed oval centrally located nuclei. Histochemical analysis of the stomach structures with AB, pH 2.5, revealed negative

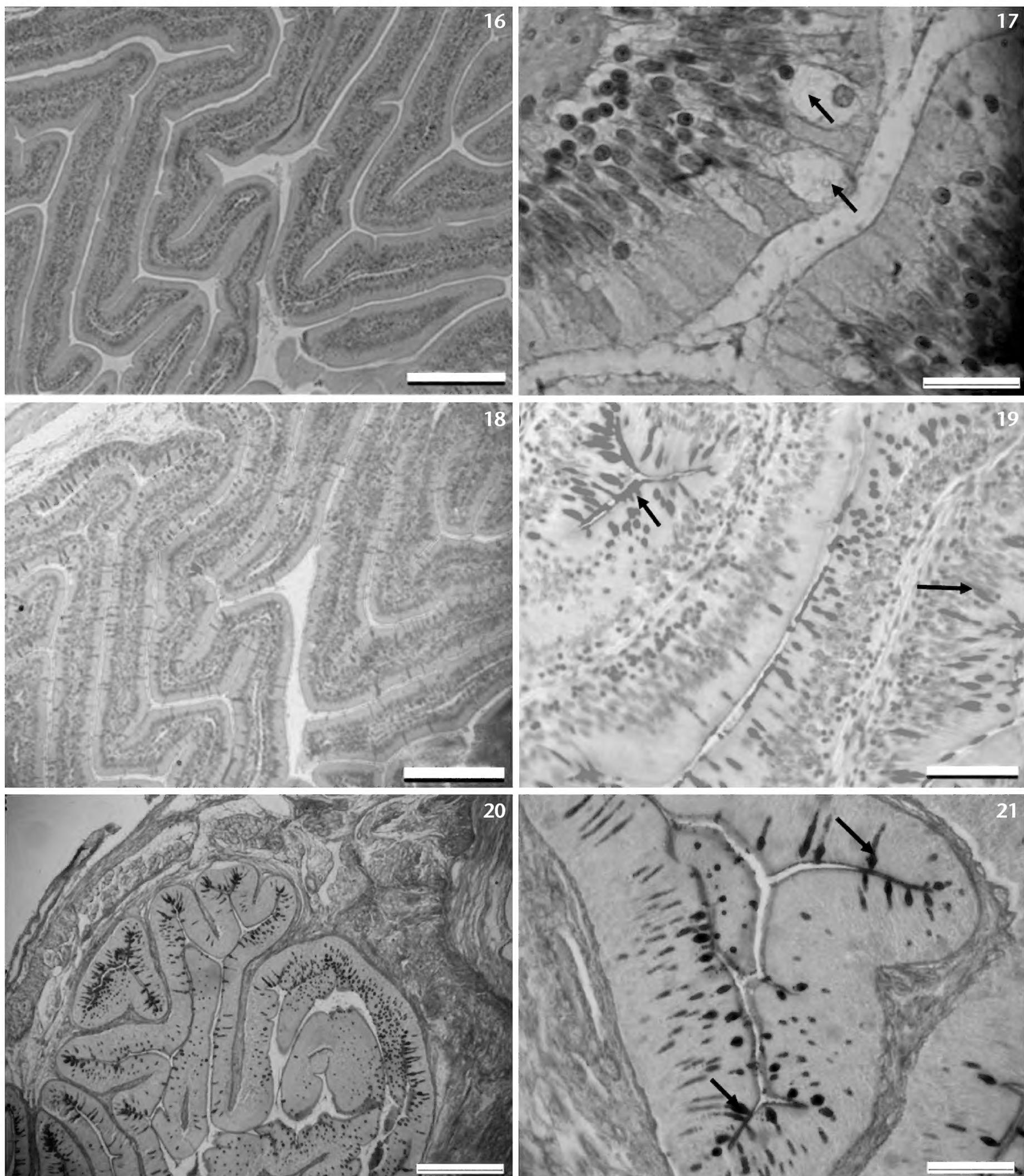
labelling of epithelial cells and gastric glands (Fig. 11) however, neck cells of gastric glands of the cardiac stomach showed moderate AB positivity indicating the presence of acidic mucins (Table 1, Figs 12, 13). Non-glandular region was AB negative. The lumen of tubular glands of the pyloric stomach was AB positive. On the contrary, apical parts of the epithelial lining cells of the cardiac region, as well as luminal cells of the gastric pits including mucus glands of the pyloric region of the stomach were observed to be rich in neutral mucins (Table 1, Figs 14, 15). Results of all histochemical staining reactions are summarized in Table 1.

#### Intestine

Typically, the mucosa of the anterior intestine was folded into long and relatively narrow branched villi (Fig. 16). Two cell types were identified in the epithelial layer of the entire intestine: the absorptive or columnar and the goblet cells (Fig. 17). Typically, goblets cells were the most prominent component of the



Figures 12–15. (12, 13) Photomicrographs of the cardiac stomach of *T. sparrmanii* showing numerous gastric glands (GG), and AB (pH 2.5) positive neck cells (arrows). (14, 15) Photomicrographs of the stomach of *T. sparrmanii* showing numerous gastric glands (GG), and PAS positive epithelial and mucous cells (arrows). Lamina propria (LP); Mucosa (M); submucosa (SM). Scale bars: 12–14 = 50  $\mu\text{m}$ , 15 = 200  $\mu\text{m}$ .



Figures 16–21. Photomicrographs of the anterior intestine (AI): (16) An overview of the anterior intestine with emphasis for zig-zag shaped villi, with columnar epithelial cells, which are well endowed with goblet cells. H&E stain. (17) Shows an enlarged image of a segment of Fig. 16 highlighting epithelial lining of the anterior intestine endowed with goblet cells (arrows). H&E stain. (18, 19) Highlights epithelial cells of the anterior intestine endowed with AB (pH 2.5) positive goblet cells. Note the elongated and oval shaped positive cells (arrows) in Fig. 19. (20, 21) Highlights epithelial cells of the anterior intestine endowed with PAS positive goblet cells. Note the teardrop shaped PAS positive cells (arrows) in Fig. 21. Scale bars: 16, 18, 20 = 200 µm, 17 = 20 µm, 19, 21 = 50 µm.

intestinal epithelium and were sandwiched between epithelial columnar cells. Here, they were flask shaped with two main parts; the swollen apical and the elongated basal cells. The lamina propria consisted of loose connective tissue that penetrated and supported the villi. The sub-mucosa was thin and lied above the lamina propria without a separating line in some instances (Figs 16, 17). Goblet cells at the tip of the villi of the anterior intestine showed strong labelling with AB pH 2.5 (Table 1, Figs 18, 19) and strong PAS reaction. (Table 1, Figs 20, 21).

The mucosa of the mid-intestine was made of short longitudinal unbranched folds similar in structure to those seen in the anterior intestine except that the mid-intestine had relatively few but larger goblet cells (Figs 22, 23). The brush border formed a continuous layer above the columnar epithelium and was interrupted by the goblet cells. Goblet cells of the mid-intestine segments of the intestine revealed moderate labelling with both AB (pH 2.5) and PAS stains (Table 1, Figs 24, 25).

The posterior intestine was characterized by shorter intestinal transverse villi (Table 2), with numerous goblet cells. In some segments there were hardly villi, making the folds thicker; some being leaf shaped, with no apparent distinction between the lamina propria and the sub-mucosa (Figs 26, 27). From the mid-intestine towards the ileum the number of goblet cells increased tremendously and acquired an oval to round shape unlike those seen in the anterior segment of the intestine. It was also determined that the length of microscopic folds (villi) of the intestine decreased progressively from the anterior intestine to the posterior segments. Results of the thickness of tissue layers forming the wall of the intestine in *T. sparrmanii* are displayed in Table 2. The longest villi being detected in the anterior segment ( $p < 0.0001$ ) and the largest villus diameters in the posterior intestine ( $p < 0.0001$ ).

Table 2. Thickness ( $\mu\text{m}$ ) of tissue layers forming the wall of the intestine in *T. sparrmanii*.  $p < 0.001$ .

Intestinal segment	Muscle tissue layer	Villi width	Villi length
Anterior	$105.3379 \pm 9.884823^{\text{a}}$	$108.5855 \pm 5.959744^{\text{a}}$	$625.9519 \pm 42.06772^{\text{a}}$
Mid-intestine	$38.20750 \pm 2.045053^{\text{b}}$	$118.1115 \pm 6.403478^{\text{a}}$	$368.8625 \pm 11.84932^{\text{b}}$
Posterior	$58.26950 \pm 3.524109^{\text{b}}$	$214.1985 \pm 13.67820^{\text{b}}$	$155.7320 \pm 9.017996^{\text{c}}$
p	*	*	*

Means in the same column with different superscripts are statistically different as demonstrated by one way ANOVA followed by Scheffe's test ( $p < 0.0001$ ).

In all intestinal segments, the muscular layer had same basic organization, with an internal circular and external longitudinal muscle layers. Unlike the anterior and mid-intestine, the posterior intestine had thick muscle fibres. As in the anterior region, the distal intestine had layers of nervous tissue between the muscular layers. The serosa of the intestine was very thin with squamous epithelium. The cells were flattened with scant cytoplasm and a compressed oval nucleus. Goblet cells of the distal intestine revealed moderate AB (pH 2.5) labelling (Table 1, Figs 28, 29) and a strong reaction with PAS (Table 1, Figs 30, 31).

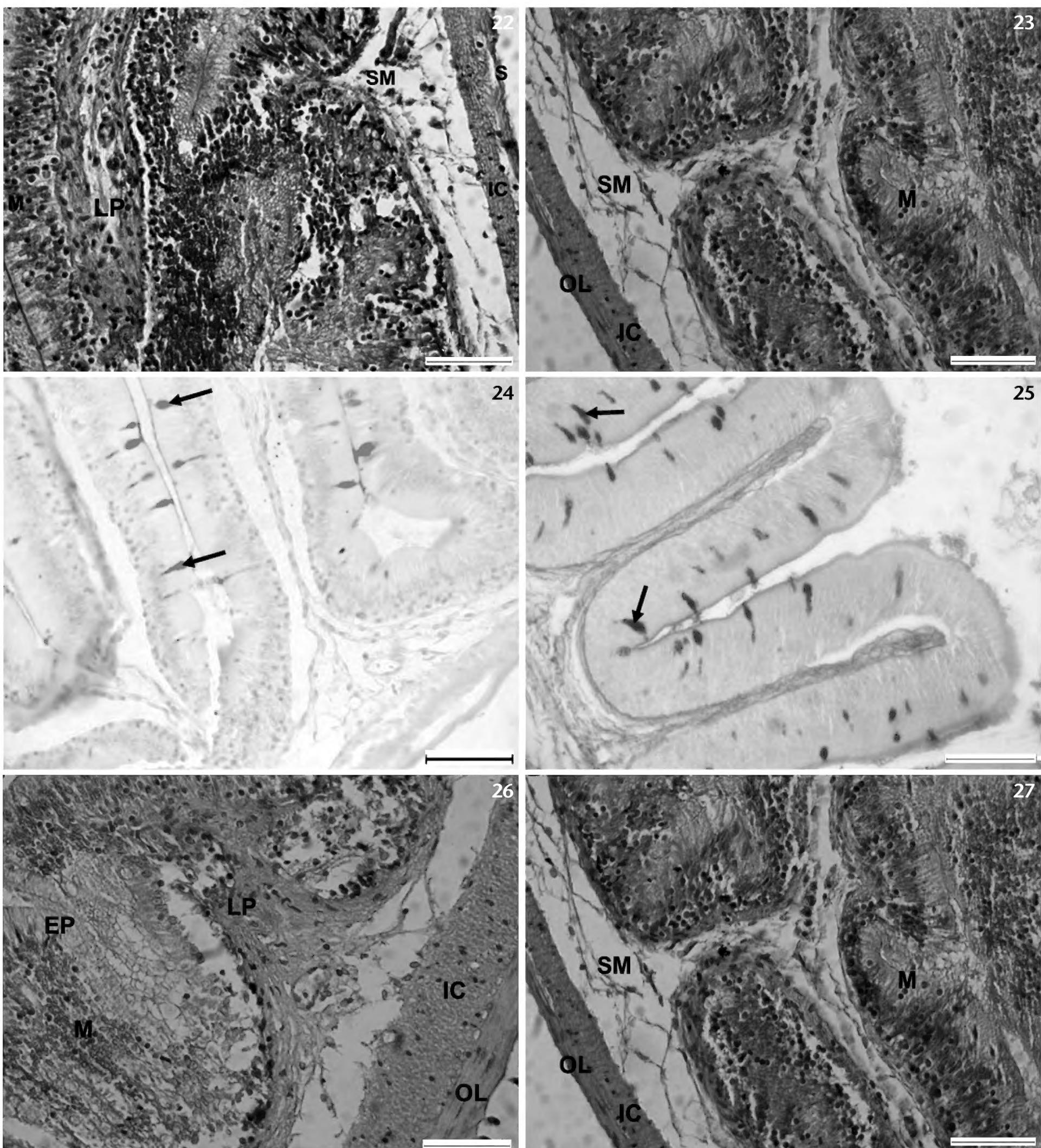
## DISCUSSION

Biodiversity in tropical freshwaters ecosystems is very high however; many tropical fish species are yet to be described. For most of these tropical fish, basic baseline morphological information is very scant in the literature. This necessitated among others histological and histochemical characterization of GIT of *T. sparrmanii*.

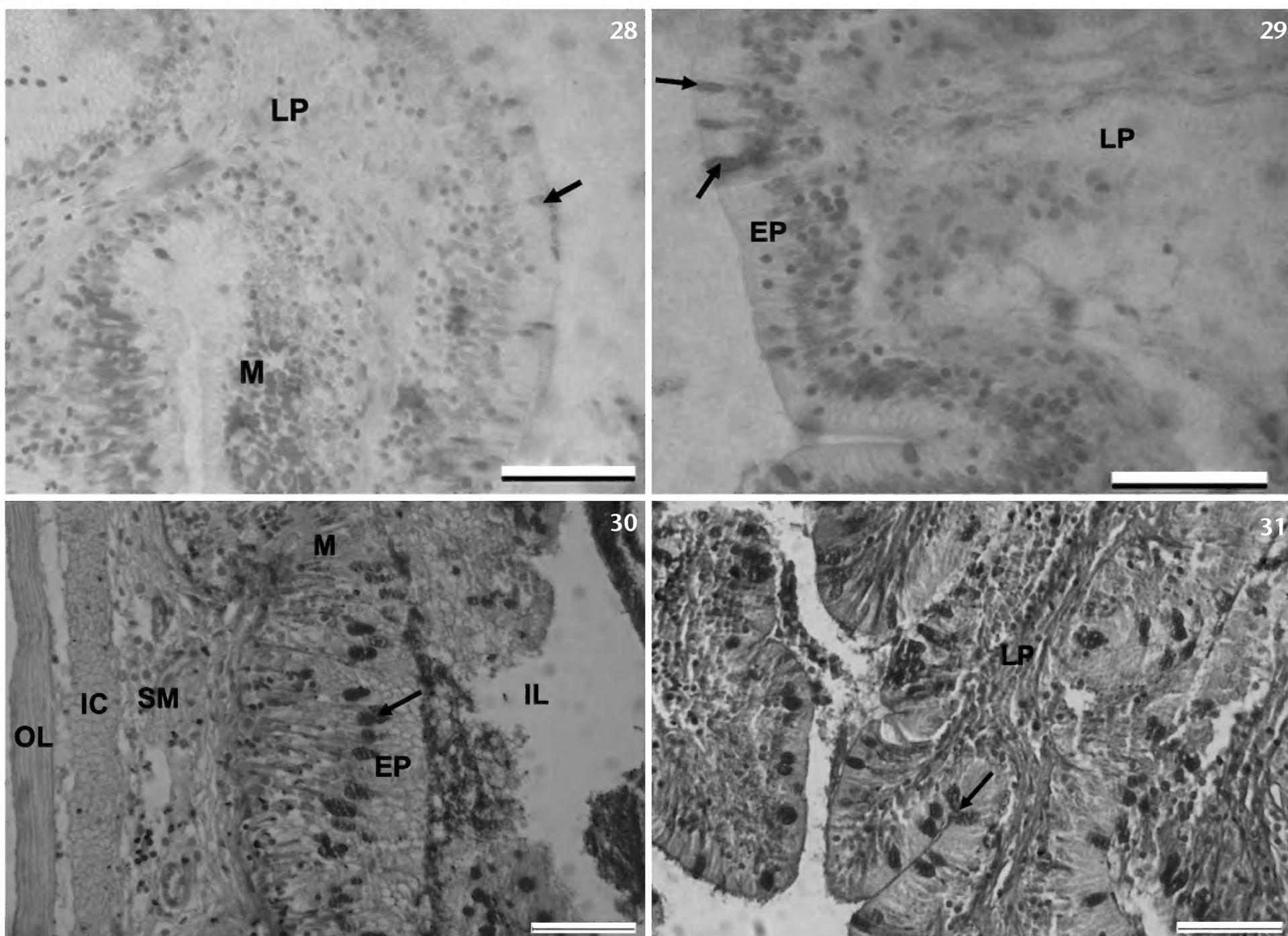
Morphological characterization of the GIT in fish is essential to understanding the biology of species under various physiological and pathological conditions. The GIT of fish display remarkable difference in their morphology and functions however, as is in the current study, the stomach and intestinal walls in many teleost fish exhibit four distinct layers; the mucosa, sub-mucosa, muscular and serosa.

A thick layer of connective tissue was a characteristic feature of oesophagus of *T. sparrmanii*. Connective tissues aid in maintaining oesophageal wall integrity as reported in other fish species (AL-Abdulhadi 2005). The long branched folds of mucosal layer of the oesophagus as seen in the current study aid in increasing the organs capacity for distention during food translocation an effect described in other teleost fish by many researchers (Santos et al. 2007, Legler 1993, Zug et al. 2001). The presence of stratified epithelium with large mucus and goblet cells observed in the current study are similar to those described in the oesophagus of other teleost fish such as the Nile tilapia, *Orechromis niloticus* (Linnaeus, 1758); white sturgeon, *Acipenser transmontanus* Richardson, 1836; rice field eel, *Monopterus albus* (Zuiw, 1793); common dentex, *Dentex dentex* (Linnaeus, 1758); and the mud loach, *Misgurnus mizolepis* Günther, 1888 (Gargiulo et al. 1996, Domeneghini et al. 1999, Morrison and Wright 1999, Park and Kim 2001, Carrasson et al. 2006, Dai et al. 2007). It is within these epithelial tissues that cell renewal occurs, which is essential for mucosal layer maintenance, protection from the invasion of pathogens as well as mechanical abrasions (Humbert et al. 1984, AL-Abdulhadi 2005, Díaz et al. 2005).

The presence of PAS positive cells in tissues often indicates the presence of neutral mucins, whereas AB (pH 2.5) positivity indicates presence of mucins with carboxyl groups. The presence of these two types of mucous producing cells in the oesophagus as seen in the current study has been reported in other fish such as the Pike (Petrinec et al. 2005) and the Tubenose goby (Wołczuk et al. 2015). Domeneghini et al. (1998) and Leknes (2011) suggested that the co-existence of cells secreting both neutral and acidic mucins represents the sequential nature of mucous biosynthesis with cells producing neutral mucins only (PAS-positive) representing an earlier stage in development compared to the other cell types. Commonly, the oesophagus of carnivorous and omnivorous fish such as *T. sparrmanii* as seen in the current study, contains numerous mucus cells while, its number decreases in herbivorous and piscivorous fish (Reifel and Travill 1978), with exceptions, for example in the *T. spilurus* with herbivorous diets but with numerous mucous cells (AL-Abdulhadi 2005). Presence of acid mucins in these cells in



Figures 22–27. Photomicrographs of the middle and posterior intestine of *T. sparrmanii*. (22, 23) Photomicrographs of transverse sections of the middle intestine, showing mucosa, (M); lamina propria, (LP); submucosa, (SM); internal circular muscular layer, (IC); external longitudinal muscle layer, (OC) H&E stain. (24) Photomicrographs of transverse sections of the middle intestine, showing AB (Ph 2.5) positive cells (arrows). (25) Photomicrographs of transverse sections of the middle intestine, showing PAS positive cells (arrows). (26, 27) Photomicrographs of transverse sections of the posterior intestine, showing mucosa, (M); lamina propria, (LP); submucosa, (SM); internal circular muscular layer, (IC); external longitudinal muscle layer, (OC); serosa (S) and the epithelium (EP) H&E stain. Scale bars: 50 µm.



Figures 28–31. Photomicrographs of the middle and posterior intestine of *T. sparrmanii*. (28, 29) Photomicrographs of transverse sections of the posterior intestine, showing AB (Ph 2.5 positive cells (arrows). (LP), lamina propria; (M), mucosa; (EP), epithelium. (30, 31) Photomicrographs of transverse sections of the posterior intestine, showing PAS positive cells. PAS/haematoxylin stain. (LP), lamina propria; (M), mucosa; (EP), epithelium.

*T. sparrmanii* may also confer high viscosity to the mucus, which would be essential for rapid and consistent lubrication of food particles during swallowing and also aid in trapping small particles and protecting the epithelium against bacterial and viral infections as reported for other fish species (Kozarić et al. 2007, Diaz et al. 2008). Acid mucins may also influence the effect of neutral mucins by creating an optimal chemical environment for digestive functions (Oliveira-Ribeiro and Fanta 2000, Abd El Hafez et al. 2013).

The presence of neutral mucins in the oesophagus of *T. sparrmanii* as in other fish species may facilitate food transport into the stomach and protect the oesophageal wall from mechanical damage including participation in enzymatic food digestion and its transformation into chime (Grau et al. 1992, Domeneghini et al. 1998, Abd El Hafez et al. 2013). The main function of the oesophagus in fish is the transfer of food however; the presence of numerous large mucous cells in the

epithelium as well as small goblet cells as seen in the current study seems to suggest the possibility of pre-gastric digestion by the oesophagus. This finding has been reported by other authors (Reifel and Travill 1978, Murray et al. 1994, Diaz et al. 2003). Digestive capacity of the oesophagus is also related to the volume of the folds in the mucosa, with a great number of folds implying more efficient digestion. The transition from the esophagus to the stomach was characterized by abrupt change from stratified squamous epithelium to simple columnar epithelium, which is a phenomenon previously reported in some teleost fish (Grau et al. 1992, Murray et al. 1994, Oliveira-Ribeiro and Fanta 2000, Dai et al. 2007, Ikpegbu et al. 2013).

When present, the main function of fish stomach is that of food storage and production of hydrochloric acid (HCl) to aid digestion. Morphologically the stomach in *T. sparrmanii* has the same basic organization in terms of cell types as in most teleost fish (Gosh and Chakrabarti 2015a, 2015b), in-

cluding other cichlid fish (Caceci and Hrubec 1990, Gosh and Chakrabarti 2015a, 2015b). The prominent cardiac region with its compact gastric glands confirms the role of stomach in gastric digestion. These results are concurrent with those reported for other fish (Gosh and Chakrabarti 2015a, 2015b). Similarly, tubular systems have been reported in mammalian peritatal cells, which are responsible for hydrochloric acid (HCl) secretion in the stomach (Bloom and Fawcett 1986). Gargiulo et al. (1997) reported that oxytic cells are responsible for acid production and their secretion play a vital role in regulating the pH of the gastric lumen. Secretion of acid sulphated mucins in the sub-epithelial portion of the stomach in *T. sparrmanii* is similar to those reported in *Oreochromis* hybrids by Caceci et al. (1997) and Gargiulo et al. (1997). Secretion of HCl is specifically necessary in these fish for algal and detrital bacteria digestion. (Chakrabarti et al. 1992, 1995). The presence of acidophilic granules in neck cells of gastric glands as seen in the current study may imply presence of mast cells, which usually mediate immune responses to pathogens (Agius and Roberts 2003). The elaborate folding in the stomach mucosa as seen in the current study may be an adaptation to increase surface area for chemical digestion and also the expansion of the organs diameter for storage of large volumes of food (Legler 1993). Alternatively, mucosal folds may play a role in delaying food passage through the stomach into smaller portions to maximize mixing food with digestive enzymes.

The strong PAS reaction by columnar epithelial cells in the stomach in *T. sparrmanii* suggests the predominance of neutral mucins in epithelial cells along the mucosal border of the stomach. Neutral mucins serve to protect mucosal surface against microorganisms and high acidity of the stomach contents. This is in agreement with the findings of Domeneghini et al. (1999) in the white sturgeon, Grau et al. (1992) in the Atlantic blue tuna, and, Morrison and Wright (1999) in *O. niloticus*. Mucus secreting cells in the mucosa as mentioned above may also aid passage of food as well as adjusting the pH as reported by Osman and Caceci (1991) in *O. niloticus*. In comparison to the other regions of the stomach, surface mucus cells were uncommon in the pyloric region of the stomach. This is indicative of a 'food retentive' more than a 'food digestive' function for this stomach region. The storage of food in the pyloric region (aided by the pyloric sphincter) before it enters the intestine would allow more time for digestion as has been observed in other fishes (Raji and Norouzi 2010). Here, mucus cells seem to protect epithelial cells (Anderson 1986), thereby allowing passage of rough particles to pass along the GIT while striated muscle seen in this region would allow voluntary regurgitation possible. Otherwise, it is accepted that the glandular region of the stomach has digestive functions while the non-glandular region acts to carry food to the gut with the help of epithelial secretions and the muscular layer. This would be in conformity with the argument of Al-Hussaini (1953). The role of a sphincter like ridge (valve) characterized by the presence of striated muscles seen between

the stomach and the proximal intestine probably prevents food from passing to the intestine without chemical digestion as suggested by Morrison and Wright (1999).

Structurally, the intestine in *T. sparrmanii* is similar to those described for other fish. However, the intestine is highly coiled, a characteristic which is usually seen in fish with herbivory diet. The highly coiled intestine has been described in other fish species and is believed to aid in absorption processes (Murray et al. 1994). Mucosal folds would be ideal for reducing speed of intestinal traffic assuring efficient absorption, thus aiding efficient food utilization (Khana and Mehrotra 1971). Intestinal length and the number of villi increase the number of enterocytes. Enterocytes are often attached to each other by tight junctions. Together enterocytes and tight junctions form a continuous barrier that regulates both transcellular and paracellular diffusion of molecules, thus constituting the principal component of the intestinal primary barrier (Murray et al. 1994, Takashima and Hibiya 1995, Beck and Peatman 2015). Intestinal villi in *T. sparrmanii* as in other teleost fish such as the Atlantic halibut, yellow flounder, rainbow trout and channel catfish favour absorptive role.

In the current study absorptive cells decreased in size towards the distal intestine, whereas goblet cells increased in size and number from the mid-intestine towards the posterior. The gradual increase in the number goblet cells from the anterior to the posterior region of the intestine has been reported in a number of fish species with different feeding habits including cichlids (Tibbetts 1997, Chirde and Gadlikar 2014). On the contrary in *T. sparrmanii* and other fish such as *Onchorynchus mykiss* (Walbaum, 1792) there is a high number of goblet cells in the anterior intestine as well, which demonstrated a strong reactivity to AB (pH 2.5) and PAS stains. Strong presence of acid mucins in the anterior intestine may imply the need for increased viscosity, which is associated with the lubrication of the intestinal mucosa and further propulsion and lubrication of undigested food towards the middle and posterior intestines. However, staining intensity with AB (pH 2.5) decreased from the mid intestine to the posterior. The high number of goblet cells towards the rectum seems to be a universal feature in most fish species and is probably useful for increased mucous production to safeguard the intestinal lining and aid faecal expulsion as previously reported by Murray et al. (1996) and Dai et al. (2007).

In conclusion, the functional structure of the GIT in *T. sparrmanii* was investigated and considered as an adaptation to its omnivore feeding behavior (having a small stomach and a long intestine). The histo-architecture and the morphology of the different regions of the GIT, including the histochemical features of the various cells lining the alimentary canal has been highlighted. These results will provide background information on gross morphology as well as some functional components of the GIT in *T. sparrmanii*, which are essential for the generation of data for the fish under investigation. However, further detailed studies are required at the ultrastructural level to confirm these findings.



## ACKNOWLEDGEMENTS

This research work was supported by a grant from the National Research Foundation (NRF, South Africa) under TTK1207112657 grant #IUD: 84357.

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 Submitted: February 11, 2020

Accepted: September 9, 2020

Available online: December 23, 2020

Editorial responsibility: Carolina Arruda Freire

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**Author Contributions:** GEO: Designed the project, captured images, drafted the manuscript and fund owner. BB: Collected and processed tissues for histology and histochemical analysis. All authors read and approved the final manuscript.

**Competing Interests:** The authors declare that there are no conflicts of interest.

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